

**Recruitment Dynamics of *Vachellia cornigera* and Its Ant Occupants in Restored Tropical
Forest**

BY

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THESIS

Submitted as partial fulfillment of the requirements
for the degree of Master of Science in Biological Sciences
in the Graduate College of the
University of Illinois at Chicago, 2018

Chicago, Illinois

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ACKNOWLEDGMENTS

I would like to thank my thesis committee – Henry Howe, David Wise and Robert Hasenstab – for their assistance in the production of this thesis, from providing feedback on the experimental design and data collection to data analysis and manuscript editing. Each committee member's expertise informed my research and is appreciated. I especially want to thank Henry Howe for advising my research and encouraging my interests, research-related and otherwise.

A number of people were involved in making my field work run as smoothly as possible, including Rosamond Coates, Benito Palacios, Isaias Landa and the staff at the Los Tuxtlas Biological Research Station. I am especially grateful to my fellow lab mate, Luis Beltran, for supporting me and keeping me motivated while I conducted my field work as well as assisting me with the development of my experimental design.

Finally, I would like to thank the members of the Howe Lab – Jason Davlantes, Crystal Guzman, Luis Beltran, Valentina Gomez and Abdel Halloway – for providing feedback on my manuscript.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOI	Area of Interest
EFN	Extrafloral Nectary
GCP	Ground Control Point
HTC	Metatibial Setal Count
HW	Head Width
MSC	Mesosoma Setal Count
MTC	Mesotibial Setal Count
NDVI	Normalized Difference Vegetation Index
PR	Pathogenesis-Related
REL	Relative Eye Length
VOC	Volatile Organic Compounds

SUMMARY

A study was conducted on the influence of environmental factors on the recruitment of the Bullhorn acacia, *Vachellia cornigera*, in experimentally restored forest habitat in Los Tuxtlas, Veracruz, as well as factors influencing the ant occupancy of this species. The abundance and height of recruited *V. cornigera* individuals located in or around the perimeter of 24 restored-forest subplots were recorded, along with the restoration treatment of the subplot and the location of each individual relative to the subplot's interior, edge or outside its edge. The Normalized Difference Vegetation Index (NDVI) was measured for each subplot as an indicator of forest canopy cover. The influence of restoration treatment, location and NDVI on *V. cornigera* abundance and height were determined. The occupancy status – occupied by *Pseudomyrmex ferrugineus*, occupied by *Pseudomyrmex gracilis* or unoccupied – of each *V. cornigera* individual was also recorded and each individual was ranked according to its estimated herbivory damage. The influence of *V. cornigera* height, *V. cornigera* location and restoration treatment on ant occupancy was determined, as well as the influence of ant occupancy on herbivory damage.

Restoration treatment was found to influence the abundance, but not the height, of recruited *V. cornigera* individuals. The greatest number of individuals was found in subplots planted with animal-dispersed tree species and the fewest were found in subplots that were not planted. Location also influenced abundance, where the greatest number of individuals were found at the edge of subplots compared to the interior and outside the edge. Canopy cover, indicated by NDVI, did not affect *V. cornigera* recruitment.

The height of *V. cornigera* individuals influenced their ant occupancy. The tallest individuals (64-500+ cm) were occupied by *P. ferrugineus*, while the intermediate-sized individuals (18-500+ cm) were occupied by *P. gracilis* and the smallest (23-107 cm) were not occupied by any ants. The location of *V. cornigera* individuals also influenced ant occupancy, where the edge of subplot hosted the highest proportions of individuals that were occupied by *P. gracilis* (69%) and *P. ferrugineus* (63%). Restoration treatment did not affect ant occupancy. Individuals occupied by *P. ferrugineus* experienced reduced herbivory damage (by around 40-44%) compared to individuals that were occupied by *P. gracilis* or were not occupied at all.

I. INTRODUCTION

A. *Vachellia cornigera* natural history

Many forms of indicators can be used to gauge the functional integrity of disturbed and restored habitat. While specific groups of organisms (plants, insects, etc.) can be used as somewhat accurate, if not comprehensive, indicators of how an ecosystem is functioning as a whole, studying community-level interactions may provide a more complete picture of the conditions of a habitat. One very well-studied interaction is the mutualistic relationship between acacia trees and acacia-ants. *Vachellia cornigera* is an acacia species that has been found growing naturally in a young restoration site (~10 years old) in Los Tuxtlas, Mexico. The recruitment dynamics of this acacia at the site, along with its interactions with ant species, both mutualistic and exploitative, can be used to determine if this community-level interaction is functioning typically or atypically in restored habitat. The purpose of this thesis is to determine if the ecology of *V. cornigera* and its ant occupants can provide an indication of the conditions of restored forest habitat. The first chapter is a literature review that introduces the broader topics of interest and relevance to the study question. The second chapter details the recruitment dynamics of *V. cornigera* at the study site and the potential factors influencing this species' recruitment and establishment, and the third and final chapter focuses on the interactions between recruited individuals of *V. cornigera* and the two ant species that were found to occupy them.

Vachellia cornigera (Seigler & Ebinger), a member of the Fabaceae family and otherwise known as the Bullhorn acacia, is one of 12 species of swollen-thorn acacias found in the tropics, from the coasts of Mexico to Colombia (Janzen, 1974). *Vachellia cornigera* is one of the most studied and

well-documented of the swollen-thorn acacias with respect to its distribution, morphology and ecology; Dan Janzen has contributed enormously to this body of knowledge. The swollen-thorn acacias are distinct from other acacias in that they have large, “swollen” thorns that are hollow, modified leaflet tips on young leaves called Beltian bodies, extrafloral nectaries (EFN) on their petioles and year-round leaf production (Janzen, 1969a; 1974; Heil et al., 2004; Rickson, 1969; 1975).

The swollen-thorn acacias are distributed within lowland Central American semi-deciduous and deciduous secondary vegetation, typically in areas with a dry season of six months or less (Janzen, 1969). *Vachellia cornigera* has the greatest geographic and ecological range of all the swollen-thorn acacias, found on virtually every soil type and in the driest sites, often co-occurring with either *V. collinsii* or *V. hindsii* depending on the moisture level of the habitat (Janzen, 1967; 1969a). It is usually found in disturbed habitat, such as creek bottoms, hilltops, pastures, roadsides, short-fallow fields and young fallow regeneration (Janzen, 1974). It does well in habitat that has been cleared of other vegetation and has become dry and insolated.

Most of the distinctive traits of swollen-thorn acacias are tailored for the mutualistic relationship they have with acacia-ants, in which ant colonies nest in the hollow thorns and feed on the nectar produced by extrafloral nectaries and the Beltian bodies (Janzen, 1974). The secreted nectar is a source of carbohydrates for worker ants and the Beltian bodies are a source of carbohydrates, lipids and proteins for the larvae (Heil et al., 2004; Rickson, 1975). The acacias in turn rely on their resident ant colonies to protect them from herbivores and encroaching vegetation, and they cannot survive to reproductive maturity without occupation of a colony of

acacia-ants (Janzen, 1967a). *Vachellia cornigera* lacks more direct mechanisms for herbivory defense, such as the use of chemical compounds as deterrents (Janzen, 1969b; Seigler and Ebinger, 1987). The acacia-ants depend on the swollen-thorn acacias for shelter and food, and ant species that are obligate mutualists cannot survive anywhere else. The dependence of acacia-ants and swollen-thorn acacias on each other makes their mutualistic relationship an obligate one.

Vachellia cornigera is distinguishable from the other swollen-thorn acacias by a combination of traits including: elongated, canoe-shaped extrafloral nectaries, elongated inflorescences, rounded and thick-walled thorns and indehiscent seed pods that must be split open by animals such as birds and cattle (Janzen, 1974). Individuals reach reproductive maturity at around 3-4 years if they are occupied by acacia-ants. Adult individuals can reach 6-18m in height and can live 15-35 years (Janzen, 1974; 1967b). Flowering in this species occurs near the end of the dry season, and the production of vegetative growth does not start again until after flowering (Janzen, 1966). Its flowers are yellow and pollinated by bees (Janzen, 1966). Seeds are 3-7mm long, 2-5mm wide and 1-3mm thick (Janzen, 1969a). *Vachellia cornigera* produces relatively few seeds in its lifetime, about 60,000, compared to other disturbance-tolerant species (Janzen, 1967b).

Birds, cattle and people have been responsible for dispersing the seeds of *V. cornigera* in secondary growth habitat. Birds eat most of the seeds before feeding on the fleshy pulp (Janzen, 1969). Seed-dispersing species that are common in secondary growth include woodpeckers, *Saltator atriceps*, *Saltator coerulescens*, *Pitangus sulphuratus* and *Psilorhinus morio* (Janzen,

1967b; 1969a). Other birds, such as orioles, break open the seed pods only to feed on the pulp inside; the seeds are not eaten, but fall to the ground (Janzen, 1969a).

Vachellia cornigera is an obligate-gap species in secondary vegetation as it requires high amounts of light to survive (Martinez-Garza and Gonzalez-Montagut, 1999). The traits of *V. cornigera* that allow it to thrive in dry, disturbed habitat make it suitable for regions like Veracruz, Mexico, where a large proportion of the remaining tropical dry forest (which represents only 7% of land area) is secondary vegetation (Williams-Linera et al., 2011). *Vachellia cornigera* is one of a few species that dominates early-successional sites. Castillo-Campos et al. (2008) found that *V. cornigera* is one of the characteristic species found in the 1-3m high shrub layer of secondary vegetation in Veracruz, Mexico. Guevara et al. (1992) found *V. cornigera* to be highly abundant in their study of pastures in Los Tuxtlas, Veracruz — it was one of the species found in >33% of their sampling quadrats. While tolerant of drier habitat conditions, *V. cornigera* cannot survive in areas with severe dry seasons; it is absent from much of eastern Mexico outside of Veracruz because the dry season in this area causes acacias to drop their leaves and thereby lose their ant colonies (Janzen, 1967; 1973).

The environmental conditions of a habitat can influence how an ant-acacia allocates investment in its growth and maintenance. The production of food bodies is especially sensitive to changes in environmental conditions and resources. Food bodies are metabolically expensive to produce because they contain proteins, especially in habitat lacking soil nutrients (Heil et al., 2004). African acacias are quite different from neotropical swollen-thorn acacias in how they respond to herbivore damage — they can increase the length of their thorns, which provides more

shelter for defending ants (Agrawal and Rutter, 1998). Mutualistic ants respond well to stress signals, released in sap or volatiles, produced by their host acacia in response to herbivory damage, where hundreds of worker ants can recruit to areas of damage (Agrawal and Rutter, 1998). The health conditions of the acacia in turn may influence its investment in its mutualistic relationship with acacia-ants. Since obligate acacia-ants depend on their acacia host for survival, their life history traits may be affected by changes in the provision of nectar or Beltian bodies caused by stressful environmental conditions (Clement et al., 2008). Ant colonies living in stressful conditions may modify how they invest in reproduction vs. the growth and maintenance of the colony.

Young seedlings of *V. cornigera* are especially vulnerable to mortality caused by herbivory damage (Janzen, 1967b). They can resist infection by phytopathogens, but are not as effective in resisting herbivory damage incurred by insects, where moth larvae and insects in the Orthoptera order have been the most harmful. *Vachellia cornigera* individuals are especially vulnerable during times of cold weather because worker ants do not patrol their host plant as often in these unfavorable conditions.

Vachellia cornigera suffers intense and frequent seed damage by bruchid beetles (*Callosobruchus maculatus*) (Janzen, 1967; 1974). Adult bruchid beetle females lay their eggs on mature seed pods, and when the larvae emerge they bore through the outer pod wall and into the seeds, where one larva will settle on each seed. Once the young adult beetles emerge from the pod, they lay their eggs on any remaining seed pods, ultimately destroying all of them (Janzen, 1969b). Species of bruchid beetles that predate on *V. cornigera*'s seeds include *Acanthoscelides*

oblongoguttatus, *Mimosestes* sp. and *Stator subaeneus*. These species destroy 20-80% of the seeds on a *V. cornigera* individual (Janzen, 1969b). The bruchid beetles are so effective at damaging seeds that likely only about 1% of a *V. cornigera* individual's seeds survive to get dispersed. A greater density of *V. cornigera* individuals is found within habitat containing vegetation of a similar age to them, and these individuals suffer greater seed damage.

Other predators of *V. cornigera* in Veracruz, Mexico are *Polyhymno* larvae, which feed on its leaves and may effectively kill the plant (Eubanks et al., 1997). These larvae are able to bypass an acacia's ant colony defense by creating sealed shelters on the plant using silk and the plant's own leaves. Ant colonies belonging to *Pseudomyrmex ferrugineus* are somewhat effective in eliminating these larvae, as evidenced in the 1997 study conducted by Eubanks et al. by the lower density and survival of the moth larvae on ant-occupied acacias and reduced herbivory damage by 50%.

And the list of *V. cornigera*'s offenders goes on. The neotropical jumping spider *Bagheera kiplingi* feed on the acacia's Beltian bodies (Meehan et al., 2009). These spiders have been found to cause damage to *V. cornigera* in southeastern Mexico and in northwestern Costa Rica. The hispid cotton rat (*Sigmodon hispidus*) also causes herbivory damage to swollen-thorn acacias (Freese, 1976; Janzen, 1967b). It was found to be an important remover of entire shoots of small (10-100 cm), unoccupied and shaded acacias in Temascal, Mexico. Birds may also cause damage to *V. cornigera* by predating on ants occupying its thorns (Janzen, 1969a). Janzen (1969a) found *V. cornigera* and *V. collinsii* individuals with their entire colonies of *P. ferrugineus* removed by birds, regardless of the protection afforded by the thicker-walled thorns

of these two species. Fires are also a stressor for *V. cornigera* in habitats where annual fires are common, such as oak-grasslands, cane fields, pastures and milpas (Janzen, 1967b). In these areas it can take 2-4 years for *V. cornigera* individuals to reappear. Regular fires destroy colonies of the mutualist ant species *P. ferrugineus*. Continuous and deliberate burning of *V. cornigera* habitat eventually eliminates this species, even if the root stock is intact.

Ants detect the quality of host acacia plants. In order to attract ants acacia plants must increase their food rewards (Agrawal and Rutter, 1998). Ants that have a facultative, and therefore more flexible, relationship with swollen-thorn acacias can move from one acacia lacking food rewards to another, even if it is a different acacia species. Some acacias that do not typically host ants are capable of recruiting defending ants via nectar secretion that is induced by herbivory damage (Gonzalez-Teuber and Heil, 2009b). Mutualistic ants are much more discerning than non-mutualistic ants in their taste for EFN nectar, preferring nectar with a higher relative concentration of amino acids to sugars that is found in ant-plants like *V. cornigera*.

The nectar produced by the EFNs of swollen-thorn acacias primarily serves to attract and maintain mutualist ant colonies by providing sugars and amino acids to worker ants, but also contains Pathogenesis-Related (PR)-proteins that protect against microbial infections (Gonzalez-Teuber and Heil, 2009b). These nectar proteins function differently from secondary compounds like alkaloids and phenols that deter organisms other than mutualistic ants from consuming nectar (Gonzalez-Teuber and Heil, 2009a). Pathogenesis-Related proteins have been shown to inhibit the growth of fungi and other phytopathogens (Gonzalez-Teuber and Heil, 2010). The prevention of pathogenic microorganisms from populating extrafloral nectar is necessary to

prevent their entry into the plant itself (Gonzalez-Teuber and Heil, 2009a). Fungi and yeast affect the sugar composition of nectar and the recruitment of acacia-ants. Gonzalez-Teuber and Heil (2009a) found that most of the proteins found in a *V. cornigera* individual's EFNs were PR-proteins, of which chitinases and glucanases made up more than 50% of the total protein content. In another study (2009b) these authors also found that the EFNs of three sympatric ant-acacias, including *V. cornigera*, were better able to inhibit the growth of phytopathogens than two acacias that do not host ant colonies that have the same distribution.

B. Mutualistic relationship between *Vachellia cornigera* and *Pseudomyrmex ferrugineus*

One of the ant species that *V. cornigera* has formed an obligate mutualistic relationship with is *Pseudomyrmex ferrugineus*. The relationship between *V. cornigera* and *P. ferrugineus* is considered to be mutualistic because it involves the exchange of resources and services between two different species that result in fitness benefits for both partners (Bronstein, 1998).

Myrmecophylia is the term used to describe plants that have a mutualistic relationship with ants, and at least 465 species of vascular plants in 52 families form a mutualistic relationship with ants in 25 genera (Jolivet, 1998).

The *Pseudomyrmex* genus includes over 200 species, and around 40 of those species are symbiotic with myrmecophilous plants (Ward, 1991; Ward and Downie, 2005; Chomicki et al., 2015). The *Pseudomyrmex ferrugineus* group, also known as the “*ferrugineus* complex”, includes 10 species that are obligate mutualists with swollen-thorn acacias (Ward, 1993). These ten species are: *P. ferrugineus*, *P. flavicornis*, *P. janzeni*, *P. mixtecus*, *P. nigrocinctus*, *P. particeps*, *P. peperis*, *P. satanicus*, *P. spinicola* and *P. veneficus*. Geographic isolation has driven speciation within this group, with different ant species evolving mutualisms with swollen-thorn

acacia species with shared geographic distributions. The relationship between the *Pseudomyrmex ferrugineus* group and swollen-thorn acacias originated in Mesoamerica between the late Miocene and the middle Pliocene and both groups diversified over the past 5 Mya in Central America (Gomez-Acevedo et al., 2010; Chomicki et al., 2015). The obligate acacia ants in the *ferrugineus* complex are not a monophyletic group; it has been determined that mutualistic behavior evolved independently multiple times in this group (Kautz et al., 2008).

Most of the species within the complex, including *P. ferrugineus*, are capable of nesting in more than one species of *Vachellia* (Chomicki et al., 2015). *Pseudomyrmex ferrugineus*, owing to its broad distribution from southern Mexico to El Salvador and Honduras, has been observed to occupy all *Vachellia* species also distributed within this geographic range, including: *V. chiapensis*, *V. collinsii*, *V. cookii*, *V. gentlei*, *V. globulifera*, *V. hindsii*, *V. janzenii*, *V. mayana*, *V. sphaerocephala* and *V. cornigera* (Ward, 1993; Gomez-Acevedo et al., 2010; Janzen, 1967b). *Vachellia cornigera* can also be colonized by various species within the *Pseudomyrmex ferrugineus* group besides *P. ferrugineus* itself, including *P. nigrocinctus*, *P. veneficus*, *P. peperii*, *P. mixtecus*, *P. flavicornis* and *P. spinicola* (Gomez-Acevedo et al., 2010).

Pseudomyrmex ferrugineus typically produces large colonies of 160 to 700 individuals (Clement et al., 2008; Kautz et al., 2012), with a maximum estimated number of 20,000-30,000 individuals (Janzen, 1973). *Pseudomyrmex ferrugineus* is a monogynous species: each colony has only one queen (Janzen, 1973). A fertilized queen that survives competitive interactions with other queens will colonize a young acacia for herself. She resides in one of the acacia's thorns (along with 75-200 worker ants) and, as the colony grows, more thorns on the acacia are

occupied by her workers and brood. Her eggs are kept in thorns adjacent to hers, where each one can contain 500-2000 eggs. Once the colony has 100-300 workers, which can take 9-12 months (Janzen, 1967a), these workers can effectively patrol and guard an entire acacia from other ant colonies and herbivores. One mature colony can occupy between one to five acacias. The production of alates, winged reproductive males and females, starts two years after the colony is founded by the queen. These alates mate in large swarms far from their parent colonies with reproductives from other colonies, and the fertilized females go on to found colonies of their own as queens. These mating swarms were observed to happen year round in southern Mexico (Janzen, 1967a).

Fertilized queens that are looking for a young, unoccupied acacia to colonize depend on a chemical signal produced by the acacias through volatile organic compounds (VOCs) (Razo-Belman and Heil, 2012). These VOCs signal the health and reward production level of a potential host acacia. Razo-Belman et al. (2017) found that high-reward acacia species produce VOC blends that actually consist of fewer compounds than those of low-reward species. These VOCs also help queens distinguish between species of acacias that are myrmecophytic, like *V. cornigera*, and those that are not (Orona-Tamayo and Heil, 2013).

Depending on mutualistic acacia-ants as their only form of defense can be risky for swollen-thorn acacias. The rewards they use to attract ant colonies (EFNs, Beltian bodies and hollow thorns) are openly available to any other organism. This can make the acacias vulnerable to exploitation by non-mutualistic organisms. As such, swollen-thorn acacias must have host sanctions in place to prevent exploitation by organisms that do not provide an anti-herbivory

service. Acacias can deter exploitative ant species from occupying them by shedding thorns or stopping the production of nectar or Beltian bodies (Orona-Tomayo and Heil, 2013). They can also alter the composition of their food rewards so that exploiter ants with a generalist diet cannot consume them – this is partner choice in action.

In addition to host sanctions and partner choice, host acacias can also conduct partner manipulation (Heil et al., 2014). Adult worker ants belonging to *P. ferrugineus* lack the digestive enzyme invertase that breaks down sucrose (Denison, 2014; Heil et al., 2005). This prevents them from consuming sucrose-containing nectar; the nectar secreted from the EFNs on *V. cornigera* do not contain sucrose. Avoidance of sucrose-containing nectar is a learned behavior, where prior consumption of extrafloral nectar from acacias leads to a preference for nectar containing only glucose instead of nectar containing both sucrose and glucose. By manipulating the diet preferences of its ant mutualist an acacia host can increase specialization of the ant (Heil et al., 2014). The larvae of *P. ferrugineus* do have invertase activity in their digestive system and are able to consume nectar with sucrose, but they are fed Beltian bodies exclusively (Kautz et al., 2009). Once a newly emerged adult worker ant feeds on extrafloral nectar her invertase activity is inhibited by the EFN protein chitinase and she becomes specialized on the sucrose-free nectar provided by her host acacia (Heil et al., 2014). In effect, increased specialization on sucrose-free nectar that is reinforced by continuous feeding on the host acacia nectar prevents occupying ants from getting their food from sources other than their host. Since their food availability is influenced by the health and conditions of their host acacia, this partner manipulation spurs the defensive behavior. Partner manipulation has been proven to be effective because *P. ferrugineus* is not observed nesting outside of its host acacia plants (Heil et al., 2004).

While adult *P. ferrugineus* individuals develop a preference for sucrose-free nectar, exploiter and generalist ant species prefer nectar that contains sucrose and are able to digest it because they have invertase (Kautz et al., 2008). Gonzalez-Teuber and Heil (2009b) observed that generalist ants have no preference in amino acids in their nectar sources, while symbiotic ants prefer higher concentrations of specific amino acids. Exploiter ants are, however, incapable of digesting Beltian bodies as effectively as the larvae of *P. ferrugineus* (Chomicki et al., 2015).

Pseudomyrmex ferrugineus typically inhabits lowland tropical vegetation that is disturbed (Janzen, 1967b), where *V. cornigera* is also distributed. Both acacia-ants and nectary-bearing plants are abundant in disturbed habitats such as forest clearings and edges (Bentley, 1976). Shade has a negative effect on the growth of the acacias and on occupying colonies of *P. ferrugineus*. Founding queens avoid shaded areas when searching for young acacia individuals to colonize. Acacias in shaded areas do not produce thorns large enough to adequately host the brood of an ant colony.

If an acacia lives in conditions that are conducive to attracting and maintaining a mutualist ant colony, the colony will likely have the resources it needs to fuel effective herbivory defense of their host. Worker ants do this through patrolling behavior on the acacia's leaves (Janzen, 1967b). Acacia individuals that are unoccupied by a defending ant colony suffer greater than 90% herbivory damage via removal of new shoots, exhibit negative growth rates and eventually die before reaching reproductive maturity. Young *V. cornigera* individuals may die after only one to two years if they are not occupied by an acacia-ant colony (Janzen, 1974).

Defending ant colonies also remove vines and other vegetation that encroaches upon *V. cornigera* individuals, which prevents the acacia from being shaded out. Suarez et al. (1998) argue that acacia-ants actually remove encroaching vegetation from their host acacias to eliminate potential bridges on which invading ants can travel.

Acacia-ants are also useful for protecting their host acacias from infection caused by fungal pathogens. Gonzalez-Teuber et al. (2014) observed that *P. ferrugineus* was much more effective in inhibiting the growth of epiphytic bacteria from *V. hindsii* via ant-associated bacteria than *P. gracilis*, an exploitative ant species. Another study conducted by Gonzalez-Teuber and Heil (2010) demonstrated that Acacia plants that have their occupying ant colonies removed experience higher microbial loads in their leaves and nectary tissues, although the extrafloral nectar does not. These plants do not have the chemical means to protect themselves from pathogens, so they depend on mutualistic ants to defend them against pathogens as well as herbivores.

Resident ant colonies, while under the influence of partner manipulation by their host acacias, are capable of modifying how they provide their services in response to their host conditions, performing their own version of partner sanctions. Ants that are not provided enough extrafloral nectar decrease their defensive activity (Orona-Tamayo and Heil, 2013). Their overall density and aggressiveness toward herbivores is hypothesized to be influenced by both the quality and quantity of secreted nectar (Sobrinho et al., 2002). Younger, pre-reproductive acacia individuals invest more in their nectar to increase ant protection of their reproductive structures.

Ant colonies hosted by *V. cornigera* increase their patrolling activity on new shoot tips instead of older foliage due to the increased production of food rewards on younger leaves (Janzen, 1967b). Gonzalez-Teuber et al. (2012) observed greater recruitment and defensive behavior towards nectar thieves by *P. ferrugineus* in response to greater investment of host acacias (*V. cornigera* and *V. hindsii*) in the concentration of extrafloral nectar. *Vachellia cornigera* invests less in growth and more in nectar production during the dry season as this is when herbivory defense is most needed. Heil et al. (2009) conducted a similar study and also found a positive correlation between nectar secretion and the activity level of residing mutualistic ant colonies. They also found that a mutualistic ant species was better able to induce nectar secretion than an exploiter species. The diversity of ant species that occupy high-reward acacia hosts is relatively low, where mutualistic ant species eventually end up dominating these host plants by replacing parasitic species (Heil, 2013). Increased nectar production favors mutualistic ants because they are better competitors for food sources. Host plants that are better defended from herbivory are able to continue to invest in the production of extrafloral nectar, thus maintaining the mutualistic relationship. Half of all species of low-reward acacias are occupied by exploitative ants, an effect of investing less in nectar.

C. Exploiters of the *Vachellia cornigera*-*Pseudomyrmex ferrugineus* mutualism

Coevolution must have occurred between the swollen-thorn acacias and obligate acacia-ants in order for their mutualistic relationship to develop and persist, especially between the common ancestor of the *P. ferrugineus* complex and its host acacia. However, coevolution has not persisted across all species within both groups since this mutualism has been established (Janzen, 1974). This has allowed for flexibility in which ant species and acacia species interact,

but has also made the acacia-*Pseudomyrmex* mutualism vulnerable to exploitation and parasitism (Ward, 1993). Low-reward acacia species that are especially vulnerable to parasitism form a derived clade within the monophyletic group of obligate myrmecophytes (Heil et al., 2009). The reason for the loose co-evolution between the *P. ferrugineus* group and the swollen-thorn acacias is that the mutualistic behavior exhibited by the ants is not inherited by newer generations – it must constantly be re-established within a colony (Wilkinson and Sherratt, 2001).

One parasitic ant species that temporarily occupies acacias is *Pseudomyrmex nigropilosa* (Janzen, 1975). Its occupancy is short-lived because the host acacia quickly succumbs to herbivory or encroaching or competing vegetation, or is subsequently occupied by a colony of obligate acacia-ants. In cases where a colony of *P. nigropilosa* occupies a host acacia that is also occupied by a colony of an obligate acacia-ant, growth of the mutualist colony may slow. The life history of *P. nigropilosa* reflects its short-term occupancy strategy: this species produces alates early in colony development and invests more in the production of the brood relative to workers.

Another known parasite of acacias, *Camponotus planatus*, differs from *P. nigropilosa* because they are aggressive and compete directly with obligate acacia-ants (Janzen, 1974). Workers of this species are effective in patrolling their host plants, protecting them from herbivores. However, Raine et al. (2004) observed that *C. planatus* does not defend its host plants from herbivorous insects. It is a prolific parasite that obtains food from many species within the Leguminosae family (which contains the swollen-thorn acacias) (Rico-Gray, 1993). Raine et al. (2004) observed that colonies of *C. planatus* co-inhabit mature *V. mayana*

individuals with colonies of *P. ferrugineus*, and it appears they do not prefer to occupy saplings smaller than 1 m in height. *Camponotus planatus* consistently outcompeted *P. ferrugineus* for access to EFNs in this study. These two ant species have also been observed co-occupying *V. cornigera* and *V. hindsii* in Guatemala, where the preference of *C. planatus* colonies for mature trees persists (Janzen, 1974). In addition to these parasitic ant species, a variety of arthropods, including thrips, salticid spiders and microlepidopteran larvae, have been found to occupy thorns of *V. cornigera* in Mexico that are also occupied by colonies of obligate acacia-ants (Janzen, 1967b). Other species of non-mutualistic ants also found occupying acacias include those belonging to the *Crematogaster*, *Camponotus*, *Zacryptocerus*, *Solenopsis* and *Pseudomyrmex* genera.

The non-mutualistic ant species of interest in this thesis is the generalist exploiter *P. gracilis*. Exploiter ants do not evolve from mutualistic ancestors (Chomicki et al., 2015). They are capable of taking up residence in a variety of nesting habitat types. The ancestor of *P. gracilis* was also a generalist species, and within this species group exploitation has evolved in both *P. gracilis* and *P. nigropilosus* (Kautz et al., 2008). Neither of these species is closely related to the *P. ferrugineus* group. These two species have independently evolved facultative occupancy of swollen-thorn acacias.

Pseudomyrmex gracilis is a large, slender arboreal ant that has a broad distribution ranging from southern Uruguay and central Argentina up to southern Texas and southern Louisiana (Wetterer, 2010). It has also been introduced to Florida and Hawaii, making it the first species within the Pseudomyrmecinae subfamily to establish populations outside of its native

range. It exhibits much phenotypic variation across its geographic and ecotypic distribution, and even within individual colonies (Ward, 1993). This phenotypic variation encompasses variation in size, but more in color, where individual *P. gracilis* ants can be either mono- or bi-colored from orange to black. *Pseudomyrmex gracilis* can be found a diverse range of habitat types, including mangroves, thorn scrub and rainforest, but is especially common in disturbed habitat such a roadsides and secondary forest. They normally build their nests in dead twigs or small branches, but can also be found occupying swollen-thorn acacias throughout Central America.

Pseudomyrmex gracilis ants, being generalists, are able to obtain carbohydrates through either extrafloral nectar or other sources of sugar (Aguirre et al., 2013). This species has been found to occupy *Vachellia* plants and, by doing so, preventing founding queens of obligate-acacia ant species from colonizing the same plants (Chomicki et al., 2015). *Pseudomyrmex gracilis* workers are not aggressive towards unrelated worker ants of the same species, allowing multiple colonies with multiple queens to occupy one host acacia (Kautz et al., 2012). However, workers from different acacia trees do behave aggressively towards ants from another tree, probably due to the different cuticular hydrocarbon signatures these spatially separated colonies carry. Colonies of *P. gracilis* have also been found to co-occupy acacia trees with other non-mutualistic species, such as *P. nigropilosus* (Kautz et al., 2009).

Occupancy of a host acacia by *P. gracilis* has a negative effect on the acacia's growth. This species does not fulfil the defensive role required by acacias and also prevents mutualistic ant colonies from fulfilling this role as well (Clement et al., 2008). Clement et al. (2008) found that *V. hindsii* plants occupied by *P. gracilis* have more dead shoot tips and experience higher

levels of herbivory and vegetative overgrowth than plants occupied by *P. ferrugineus*. Gonzalez-Teuber et al. (2013) compared the effect of occupancy by *P. gracilis* or *P. ferrugineus* on protection of *V. hindsii* against pathogens and found that bacterial abundance and pathogen-caused leaf damage was higher for plants occupied by *P. gracilis*. As is typical of exploiter ant species, *P. gracilis* colonies are relatively small and consist of a higher proportion of reproductive individuals (Clement et al., 2008). In comparison to colonies of *P. ferrugineus*, *P. gracilis* workers do not actively patrol their host plant and exhibit avoidance behavior when their host is being attacked. They do, however, attack intruding *P. ferrugineus* workers and queens on an acacia that they've originally colonized (Clement et al., 2008). If placed on acacia plants originally colonized by *P. ferrugineus*, *P. gracilis* ants will demonstrate evasive behavior, and can be quite effective in avoiding being killed. It appears that *P. gracilis* ants are only conditionally aggressive.

Pseudomymrex gracilis can occupy an acacia without needing to defend it from other organisms because it does not depend on its host acacia as its sole food source (Clement et al., 2008; Kautz et al., 2012). Workers have been observed to accept pieces of ham, egg and dead insects as alternative sources of protein to Beltian bodies. Stable isotope analysis and behavioral studies have shown that the diet of *P. gracilis* colonies consists of a greater proportion of alternative food sources than that of *P. ferrugineus* (Clement et al., 2008).

Host sanctions, such as reduced food reward production, can be induced by the presence of *P. gracilis*. The Beltian bodies of acacias also contain protease inhibitors that reduce the digestive capacity of exploiter ant larvae that are not adapted to this particular food source

(Orona-Tamayo et al., 2013). *Camponotus planatus* larvae also lack the ability to digest Beltian bodies, making this food reward more resistant to exploitation than extrafloral nectar (Raine et al., 2004).

The natural history of *V. cornigera*, the mutualistic relationship between *V. cornigera* and *P. ferrugineus* and the exploitation this relationship by other ant species such as *P. gracilis* have all been discussed in detail in this chapter. The colonization dynamics of *V. cornigera* by *P. ferrugineus* or *P. gracilis* have not been directly studied in restored forest habitat. The ant occupancy of *V. cornigera* individuals in restored habitat may directly reflect the acacia's conditions and indirectly reflect habitat conditions. The next two chapters of this thesis focus on 1) factors influencing of *V. cornigera* establishment and population demography in restored habitat and 2) acacia and habitat conditions influencing ant occupancy.

II. RECRUITMENT DYNAMICS OF *VACHELLIA CORNIGERA* IN EXPERIMENTALLY-RESTORED TROPICAL FOREST

A. Introduction

This chapter focuses on the patterns of recruitment of *Vachellia cornigera* in experimentally-restored forest plots in Los Tuxtlas, Mexico. This tree species was not planted at the study site, although it is a pioneer species that occurs in disturbed habitat such as pasture and secondary growth (Janzen, 1974). The seeds of *V. cornigera* are dispersed mainly by birds that visit secondary growth (Janzen, 1967; 1969), so its presence in the study site is indirect evidence of birds crossing open pasture to visit the restored-forest plots, where they have effectively dispersed the seeds. Not only is this species disturbance-tolerant, it can also be beneficial for disturbed habitat that is undergoing restoration because it is a legume and can fix nitrogen in the soil. The recruitment dynamics of *V. cornigera* can be an indication of how effectively the restored-forest plots are attracting seed-dispersing birds as well as the habitat conditions of the study site. The population demography of recruited acacias in the forest plots demonstrates the recruitment history of this species and how restoration treatment and other factors may affect this history. Three different treatments were used to restore the forest plots at the study site. The influence of each of these treatments on the recruitment of an animal-dispersed, disturbance-tolerant pioneer species may demonstrate which of these treatments most effectively facilitates the restoration process.

The dry tropical forest regions of the world provide ideal climate and soil conditions for serving agricultural purposes, and have thus been largely transformed into agricultural land. Veracruz, Mexico is one such region that has experienced a high rate of deforestation, where only 46 ha of forest is currently protected in comparison to the 800,000 ha of forest that once

existed (Castillo-Campos et al., 2008; Gove et al., 2005). Only 7% of the forest in Veracruz remains, and a third of that small percentage is secondary succession (Williams-Linera et al., 2011). The transformed landscape now consists of agricultural land, secondary forest and fragments of primary forest (Castillo-Campos et al., 2008). Recovery of forest habitat in areas of abandoned agriculture is possible, especially if the seeds of robust, disturbance-tolerant tree species are dispersed into these areas, setting the stage for succession. Williams-Linera et al. (2011) compared the dispersal mode of tree species found in early successional sites near forest remnants and found that animal-dispersed species fare better in both early successional sites and forest remnants than wind-dispersed or self-dispersed species. One tree species that can be found in both habitat types and dominates early successional habitat is *V. cornigera* (Williams-Linera et al., 2011; Alvarez-Aquino et al., 2014). *Vachellia cornigera* has the ability to resprout from stumps or sucker shoots; this resilience makes it especially useful for forest restoration (Guevara et al., 2004).

Vachellia cornigera is a light-demanding pioneer and early secondary tree species (Guevara et al., 1986). It is distributed in wet and dry habitat at lower elevations from southern Mexico to Costa Rica, but is mostly found in disturbed habitat (Seigler and Ebinger, 1995; Janzen, 1967b). Although it is disturbance-tolerant, it is not found in areas with prolonged dry seasons that would cause it to drop its leaves, which it needs to maintain its mutualistic ant colonies (Janzen, 1967b). *Vachellia cornigera* is an obligate-gap species when found in rainforest that has developed a canopy (Martinez-Garza and Gonzalez-Montagut, 1999). As a member for the Fabaceae family, *V. cornigera* hosts nitrogen-fixing bacteria. It is considered to have high nutritional value as feed for ruminants such as cattle due to its high N degradability

(Mota et al., 2005). *Vachellia cornigera* does well in cattle pastures because cattle grazing of the vegetation opens up the forest canopy and allows *V. cornigera* to persist (Janzen, 1967b). The seeds of *V. cornigera* can be dispersed by birds, deer, cattle and humans, although they are primarily dispersed by birds that visit secondary growth, such as *Saltator atriceps*, *Saltator coerulescens* and *Psilorhinus morio* (Janzen, 1969a).

Los Tuxtlas, Veracruz is the site of many studies on the effects of disturbance on forest habitat as well as of the dynamics of reforestation. Arroyo-Rodriguez et al. (2009) conducted an inventory of the tree species in the Los Tuxtlas reserve, which has experienced varying degrees of deforestation in different areas, and found that species in the Rubiaceae, Moraceae and Fabaceae families were most common. *Vachellia cornigera* belongs to the Fabaceae family, and was most abundant in sites of the highest level of deforestation. Castillo-Campos et al. (2008) observed that *V. cornigera* typically makes up the shrub layer of secondary vegetation that has been abandoned for greater than 10 years, and it strongly contributed to the similarity of the secondary vegetation sites studied. Mizrahi et al. (1997) conducted a study in Yucatan, another region of Mexico, in secondary forest sites that were 12 or 26 years old and found that slow-growing, later-successional species were absent from the older sites but that these sites also contained greater species richness. Light-dependent species dominated all sites, creating vegetative communities that could potentially increase soil fertility if members of the Fabaceae family were represented. While *V. cornigera* was present in both the 12 and 26 year-old sites, it was more abundant in the older sites. Martinez-Garza & Gonzalez-Montagut (1999) determined that abandoned pastures in Los Tuxtlas have high potential for reforestation via seed dispersal from nearby forest, but most species represented in the seed rain found in pastures do not

actually come from adjacent forest. *Vachellia cornigera* is known to dominate the regenerating living fences and pasture in Los Tuxtlas (Ruiz-Guerra et al., 2014). It could perform the function of a “starter species” for restoration.

B. Methods

1. Study Site

The study site is a 12-hectare area in Los Tuxtlas, Mexico consisting of active cattle pasture, living fences, isolated trees and a set of 24 experimentally-restored forest plots (NW corner: 18°35'51"N, 90°60'60"W; SE corner: 18°35'36"N, 90°06'07"W). The site is surrounded by a heterogeneous landscape of agricultural land and primary and secondary forest, and is about 1 km west of the Los Tuxtlas Biological Station belonging to Universidad Nacional Autónoma de México (UNAM) (Gonzalez-Soriano et al., 1997). The rainy and dry seasons occur June-February and March-May, respectively, with a mean annual precipitation of 4825 mm and a mean annual temperature of 25°C. The study site had been used as active pasture with planted native and exotic grasses for at least 30 years prior to restoration.

The restored forest plots were established in June 2006. The plots are arranged in a 3 x 8 grid on sloping ground from 180 to 260 m above sea level (Howe et al., 2010). The restoration plots, each 30 x 30 m, consist of three treatments: eight were planted with seedlings of 12 animal-dispersed tree species (four pioneer and eight later-successional), eight were planted with 12 wind-dispersed species and the remaining eight plots were left unplanted as the control treatment (De la Peña-Domene et al. 2013). The plots were cleared of any remnant trees before experimental restoration. Each forest plot was fenced to exclude cattle from the surrounding

pasture using three-strand fences of barbed wire attached to living poles made of *Gliricidia sepium* (De la Pena-Domene et al. 2013).

2. Acacia Observations and Measurements

Data was collected May 15-19, 2017. Each 30 x 30 m plot was divided into four subplots and observations for acacia individuals were made along the two-sided perimeter (each side 15 m in length) of one subplot as well as in the area within the subplot bounded by the two sides (225 m²) (Figure 1). The subplot surveyed for each of the plots was chosen at random. A total of 131 *V. cornigera* individuals were identified and measured.

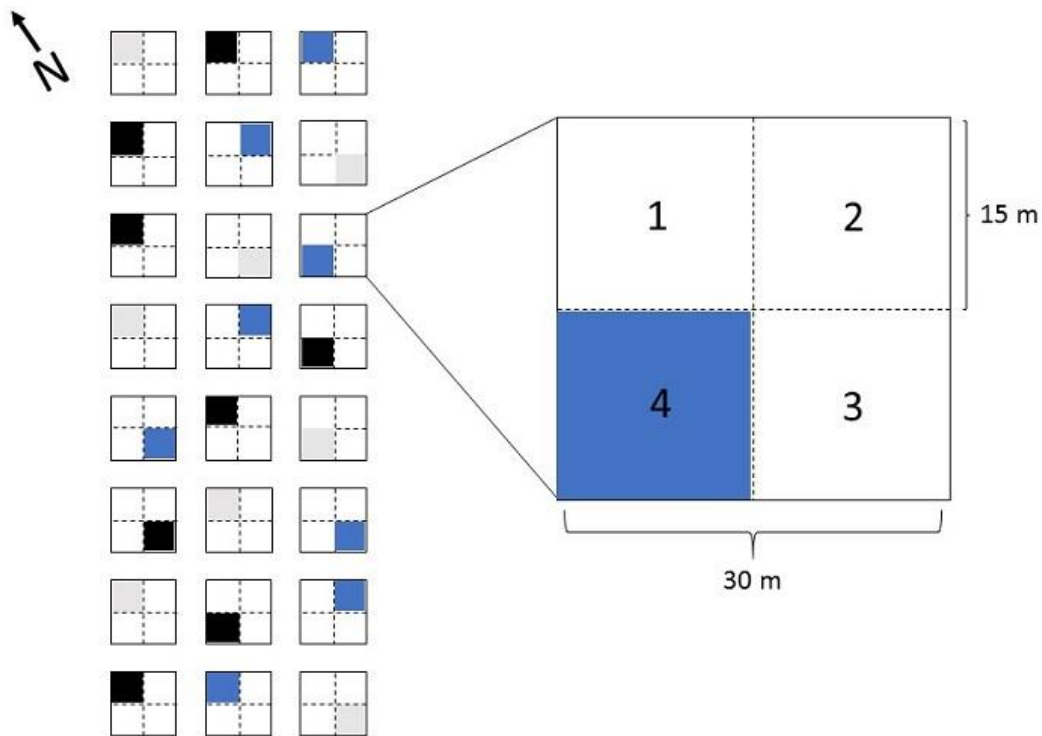


Figure 1. Layout of restored forest plots with sampled subplots color-coded according to restoration treatment (grey = control, black = animal-dispersed, blue = wind-dispersed); enlarged plot shows arrangement of subplots within each plot; figure based on De la Peña-Domene et al. (2013)

Vachellia cornigera individuals were identified by their compound leaf structure, the size and arrangement of their leaflets and the size, color and characteristic bullhorn shape of their thorns. The bullhorn acacia was the only species of acacia growing in the restored forest plots, while other species with known distributions within the Los Tuxtlas region include *V. hayessi* (Benth.) and *V. mayana* (Lundell) (Aguirre et al., 2013).

All living acacias that were encountered were included in the study. The height of each acacia individual was measured, and individuals that were greater than 2 m tall and could not be measured precisely were categorized into the following estimated height categories: >2 m, >3 m and >5 m (relabelled as 201 cm, 301 cm and 501 cm for statistical analysis). The relative location of each acacia individual was also recorded as “interior” (at least 1 m into subplot), “edge” (within 30 cm of the fence) or “outside edge” (at most 1 m outside of fence).

3. **Remote Sensing of Forest Canopy Cover**

Forest canopy cover was estimated using a remotely-sensed image of the study site from September 12 2017. The image was provided by the Pleiades-1 satellite via Apollo Mapping with a multispectral resolution of 2 m. The image was georeferenced using a polynomial rectification with ground-control points (GCPs) and a reference image of the study site from ArcGIS World Imagery (ESRI, ArcGIS online data server). A Normalized Difference Vegetation

Index (NDVI) was produced for each of the subplots (Figure 2). The index is used to determine the density of vegetation within each pixel of the image, where pixels containing forest canopy cover would produce higher NDVI values than those containing pasture or low-lying herbaceous vegetation.



Figure 2. Normalized Difference Vegetation Index (NDVI) output image of restored forest plots, with each plot and subplot outlined as an Area of Interest (AOI)

4. **Statistical Analysis**

Generalized linear models were used to determine the effect of restoration treatment and plot location (interior, edge, outside edge) on *V. cornigera* abundance. One-way ANOVA tests were conducted to determine the effect of restoration treatment and plot location on the height of recruited acacia individuals. Height was averaged for each subplot and negative square root-transformed prior to analysis. The effect of canopy cover, as indicated by subplot NDVI, on acacia abundance was analysed using a generalized linear model. A one-way ANOVA test was conducted to determine the effect of NDVI on acacia height. Finally, the effect of restoration treatment on subplot NDVI was determined using a one-way ANOVA.

C. **Results**

A total abundance of 131 *V. cornigera* individuals was observed in the sampled restored-forest subplots. The total abundance of individuals within each restoration treatment is as follows: 56 in animal subplots, 46 in wind subplots and 29 in control subplots. The animal treatment subplots had the highest average abundance of *V. cornigera* individuals (7.00 ± 2.16), while the control treatment subplots had the lowest (3.63 ± 1.43) and the wind-treatment subplots demonstrated an intermediate level of average abundance (5.75 ± 0.92) (Figure 3). The abundance of recruited *V. cornigera* individuals was affected by restoration treatment, specifically the animal treatment ($t_{21}=6.84$, $p<0.01$). It should be noted that the only subplot in which no acacia individuals were found belonged to Plot 6 (Control). This plot is estimated to have 80% of its area covered by a dense layer of invasive ferns.

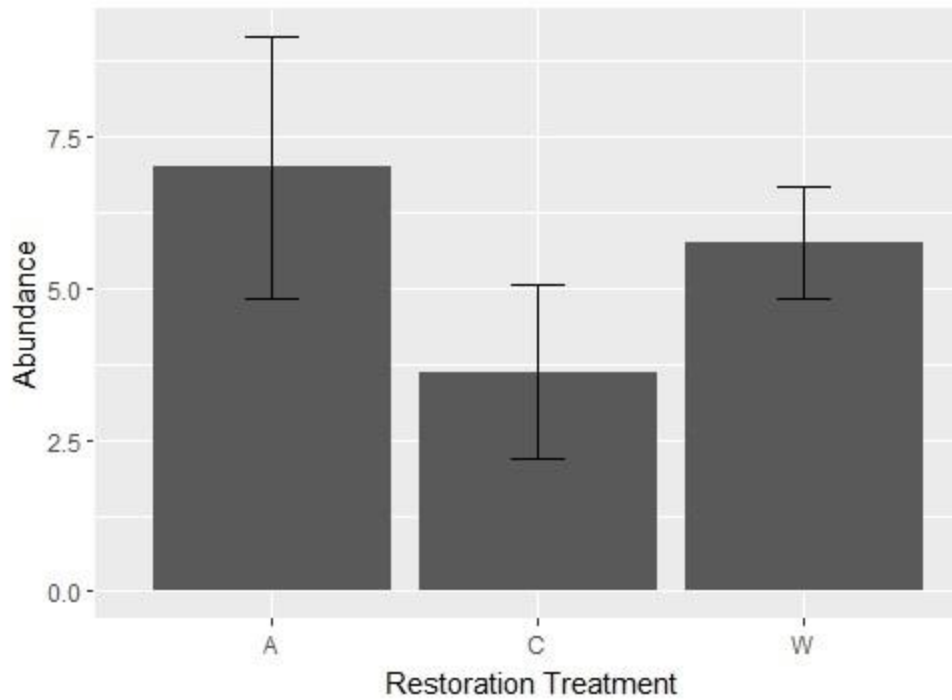


Figure 3. Barplot of mean abundance of *V. cornigera* in each restoration treatment (A = Animal, C = Control, W = Wind) with standard error bars

The distribution of *V. cornigera* height across all individuals appears to be multi-modal, where the greatest abundance of individuals were 30-60 cm and relatively large proportions of individuals were around 200 cm and 500 cm (Figure 4). Restoration treatment did not have a significant effect on the average height of recruited acacia individuals ($F_{2,20}=1.59$, $p=0.23$) (Figure 5).

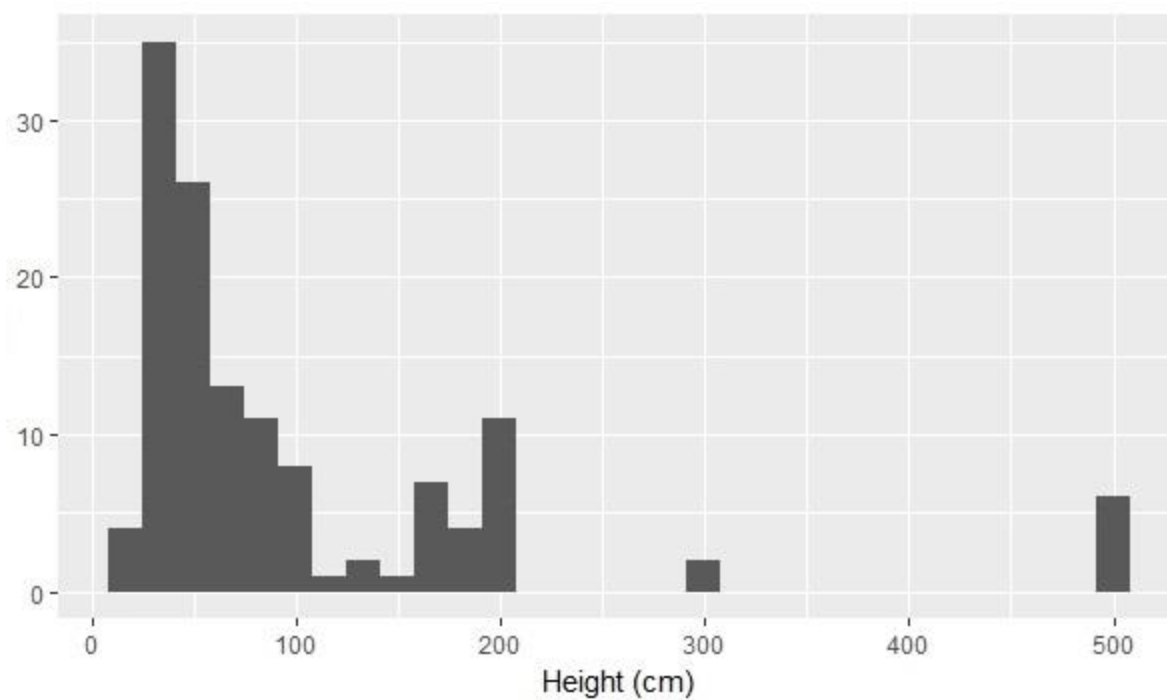


Figure 4. Histogram of *V. cornigera* abundance by height (untransformed)

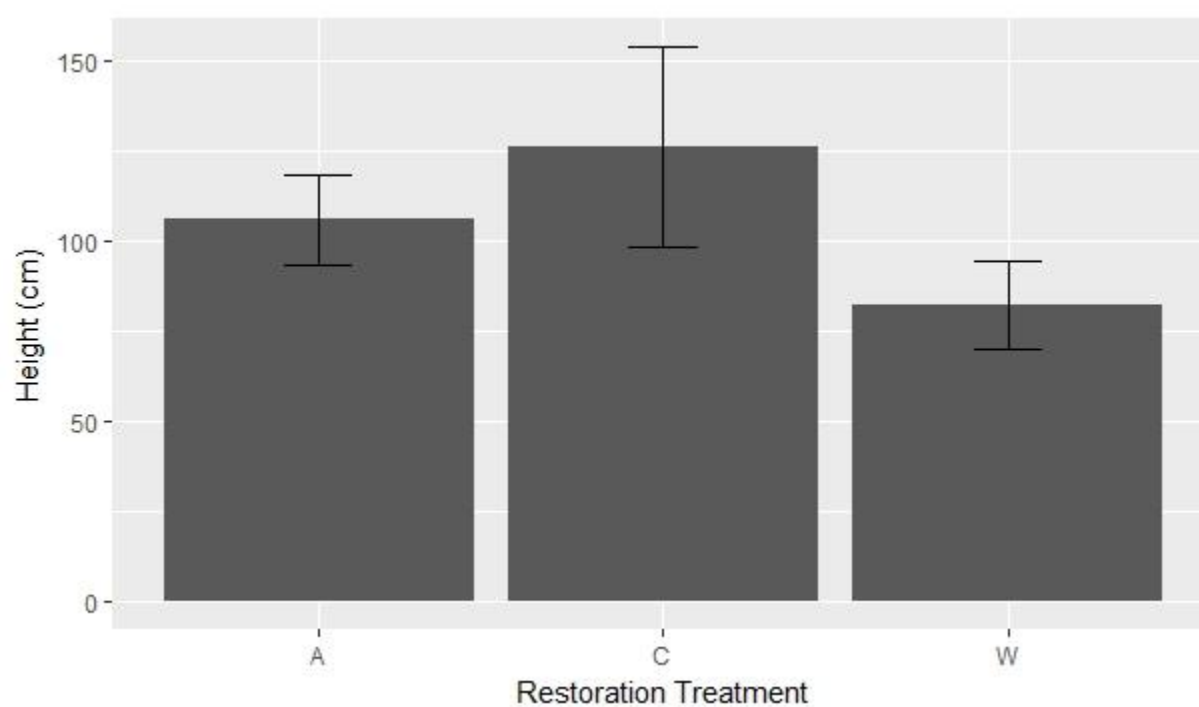


Figure 5. Barplot of mean untransformed height of *V. cornigera* in each restoration treatment (A = Animal, C = Control, W = Wind) with standard error bars

Plot location had a significant effect on the abundance of *V. cornigera* individuals (interior: $t_{69}=-3.26$, $p<0.01$; edge: $t_{69}=4.59$, $p<0.01$; outside edge: $t_{69}=-2.49$, $p=0.02$), where the average abundance of individuals within the interior of the subplots was 0.79 ± 0.39 , 3.46 ± 0.56 for the edge of subplots and 1.21 ± 0.35 for outside the subplots (Figure 6). The total abundance of acacia individuals by plot location was: 19 in the interior, 82 in the edge and 30 outside the plots. Plot location also had a significant effect on the height of acacias ($F_{2,38}=10.59$, $p<0.01$) (Figure 7)

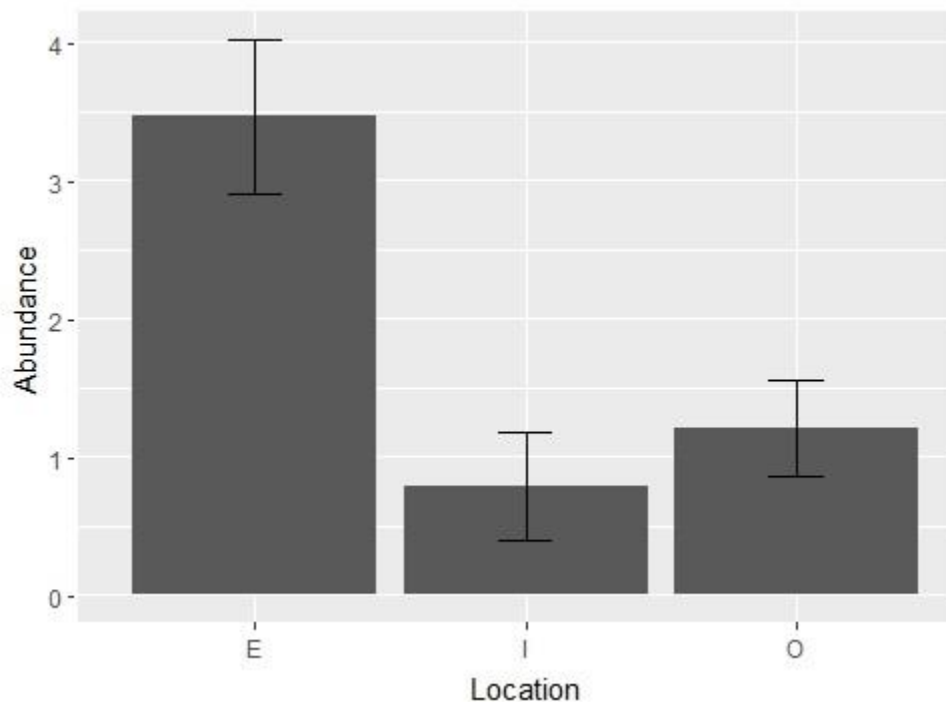


Figure 6. Barplot of mean abundance of *V. cornigera* in each location (E = Edge, I = Interior, O = Outside Edge) with standard error bars

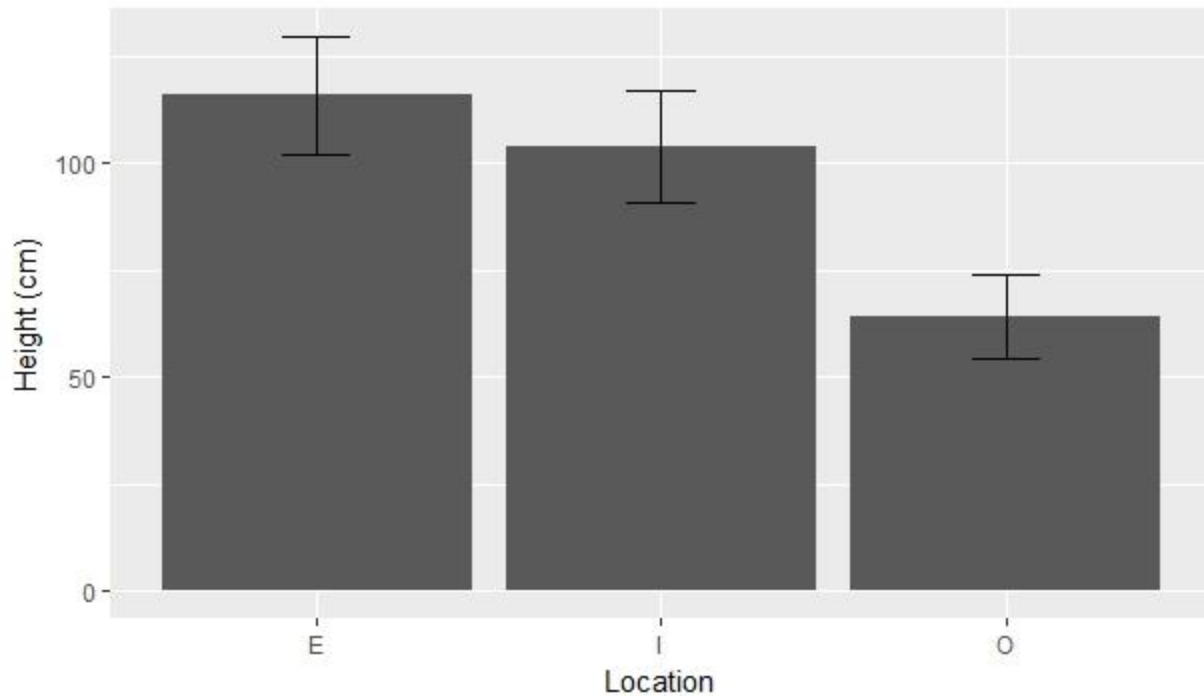


Figure 7. Barplot of mean untransformed height (cm) of *V. cornigera* in each location (E = Edge, I = Interior, O = Outside Edge) with standard error bars

NDVI values were determined for each subplot. The number of pixels assessed for the subplots range from 72 to 143. This difference in total number of pixels can be attributed to the inclusion of forest canopy growing beyond the plot fence and extending past it for some plots. The average subplot NDVI ranged between 0.69-0.75. Subplot NDVI did not have an effect on either acacia abundance ($t_{22}=-0.16$, $p=0.87$) or acacia height ($F_{1,21}=2.60$, $p=0.12$). Restoration treatment had an effect on subplot NDVI ($F_{2,21}=4.91$, $p=0.02$), where animal subplots had the highest average NDVI and control subplots had the lowest (Figure 8).

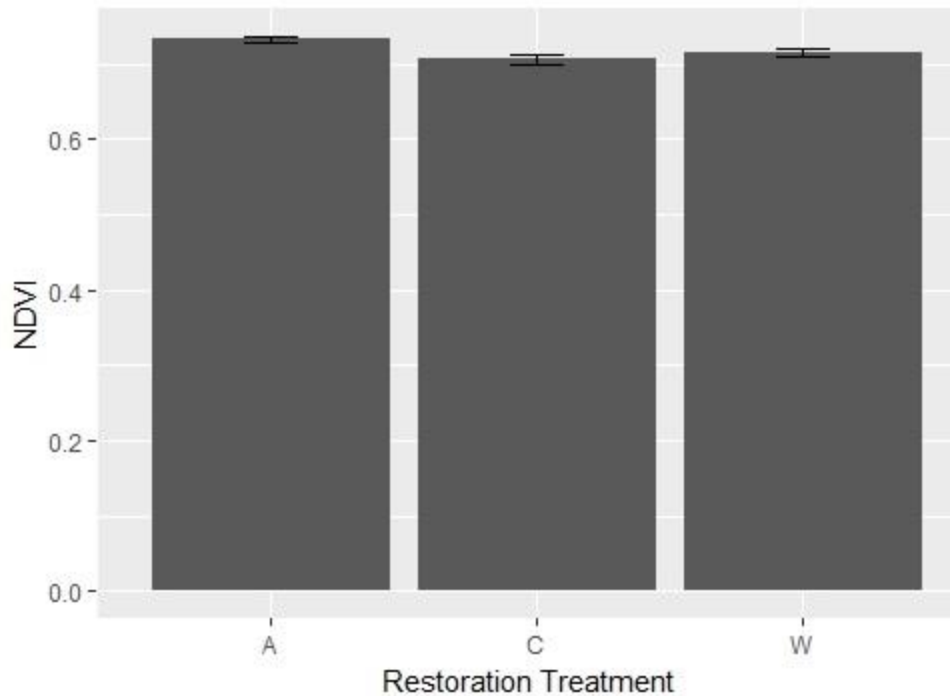


Figure 8. Barplot of mean Normalized Difference Vegetation Index (NDVI) of subplots in each restoration treatment (A = Animal, C = Control, W = Wind) with standard error bars

D. Discussion

There is evidence that restoration treatment has influenced the abundance of recruited *V. cornigera* individuals at the study site, where the greatest number of recruited individuals were in the animal subplots and lowest number of individuals were in the control subplots. The seeds of *V. cornigera* are primarily dispersed by birds, and birds are more likely to be attracted to experimental forest plots that contain animal-dispersed tree species because these species produce fruit that birds eat. The height of recruited acacias, however, was not affected by restoration treatment. The forest plots are quite close to each other and restoration treatment is randomly distributed throughout the plots in the study site. This spatial arrangement could create an environment that almost functions like stepping stones or a metapopulation of forest habitat

that facilitates the movement of seed-dispersing birds from one forest plot to another. While birds that are dispersing seeds of *V. cornigera* in the study site may spend more time in the animal treatment plots to forage, the lack of differences in the mean height of acacias in the three treatments indicates that their seeds have been dispersed in a more or less random manner over time across all subplots. These birds may be making use of the study site as one large, connected forest fragment that happens to have better foraging opportunities in certain areas. The only subplot that did not support any *V. cornigera* individuals was in a control plot that is almost entirely covered in a layer of invasive ferns that outcompete any other type of vegetation, even disturbance-tolerant pioneer tree species such as *V. cornigera*. This fern is also present in other plots in the study site to varying degrees and I suspect is a hinderance to succession in those plots where it is present.

The size class distribution of acacias in the sampled subplots indicates that *V. cornigera* has been recruiting in the study site long enough to have multiple generations established at the site. There are reproductive adults that range from 2 m to greater than 5 m tall as well as saplings near 1 m tall and many seedlings that are less than 1 m tall. Some of the smallest seedlings are likely the offspring of the reproductive adults at the site. A diverse range of size classes represented in the acacia population reflects a long history of acacia recruitment at the study site and a lack of major disturbances since the restored-forest plots have been created. Plot location influenced the height of recruited acacias, where the tallest acacias were found at the edge of the subplots and the smallest were found outside the subplots.

Location within plots also had a significant effect on the abundance of recruited acacias. The greatest number of acacias were found at the edge of subplots. An intermediate number of acacias were found outside the plots and the lowest number of acacias were found in the interior of the subplots. It is possible that there are many fewer acacias established in the interior of the forest plots because *V. cornigera* is a pioneer species that is not shade-tolerant and can therefore only establish in canopy gaps or at the edge of the plots where they have more light exposure. Acacias growing at the edge of the plots also benefit from the fences placed around each plot because they eliminate, or at the very least decrease, herbivory pressure from cattle.

Edge effects are important drivers of species interactions in fragmented forest habitat (Evans et al., 2013). Evans et al. (2013) observed that herbivory pressure from grasshoppers on a myrmecophytic plant species, *Solanum americanum*, increased with greater distance from the edge of forest habitat. The abundance of grasshoppers was correlated with higher temperatures that are present further away from the edge of forest (Evans et al., 2012). The lack of intermediate and larger-sized acacias outside the subplots could indicate that the cost of herbivory pressure is greater than the benefit of light exposure outside the plots. While many small seedlings exist outside the plots, they may not survive to reproductive age. Guevara et al. (1992) found that *V. cornigera* grows underneath the canopy of isolated trees in open cattle pasture. The acacias growing in the cattle pasture near the study site, however, are sprayed with herbicide by the owner of the pasture – an additional selective force against acacias growing in the pasture surrounding the forest plots. These smaller acacias may be the offspring of the reproductive adults growing at the edges of the plots.

The Normalized Difference Vegetation Index (NDVI) values of the forest plots in general are rather high: 0.67-0.75. Values that fall within this range are indicative of healthy forest vegetation (Meneses-Tovar, 2011), which is encouraging with respect to the progress of the 10-year-old restored-forest plots. Restoration treatment and subplot NDVI appear to be correlated. The results of this study indicate that the density of canopy cover is greatest in the animal-treatment plots and lowest in the control plots, which means that the animal-treatment plots facilitate the recruitment and establishment of trees that are contributing to the canopy layer. Tree species that can reach the canopy are likely to be later-successional species which may have their seeds brought into the study site by seed-dispersing animals from nearby forest fragments. Forest canopy cover, however, did not influence the abundance or height of recruited acacias. Birds dispersing the seeds of *V. cornigera* may prefer to visit the animal treatment subplots for the foraging opportunities rather than for cover from predators. Also, seeds brought into the interior of forest plots may not germinate or the seedlings may not survive under denser canopies due to this species' lack of shade tolerance.

Vachellia cornigera can be used as an indicator of the habitat conditions of disturbed or newly-restored sites as it is one of the pioneer species that dominates early succession (Stone et al., 2003). Since *V. cornigera* is a pioneer species, it can only reflect the ability of pioneer species like itself to recruit and establish in restored forest. The recruitment patterns of later-successional species, however, will be different, as indicated by the NDVI results from this study. The successful establishment of early-successional species is important for the progression of a forest into later stages of succession, and a healthy population of acacias can help set up the forest habitat so that later-successional species can establish under the canopy of larger acacias

and benefit from their nitrogen-fixing capabilities. Acacias that populate the edge of restored forest may also act as a buffer against herbivory with their thorns that protect against larger herbivores like cattle and their mutualistic ant colonies that protect them from herbivorous insects.

III. FACTORS INFLUENCING ANT OCCUPANCY OF *VACHELLIA CORNIGERA*

A. Introduction

The final chapter of this thesis focuses on the recruitment of ant occupants to *V. cornigera* individuals found in the experimentally-restored forest subplots and the effectiveness of the two recruited ant species, where one is a mutualist and the other is an exploiter, in defending their host acacias from herbivory. The conditions of the restored-forest habitat, and more directly the conditions of the acacia population growing in this habitat, are expected to influence which species of ants colonize the acacias. Most individuals in the population of *V. cornigera* growing in and around the restored-forest subplots are very young seedlings and a moderate number of them are reproductive adults, with lower abundances of individuals in intermediate life stages. This chapter aims to elucidate if certain conditions of the recruited acacias, such as their relative location within subplots and their life stage, influence which ant species occupies them, if any at all. The effect of ant occupancy on herbivory damage to the acacias is also considered.

Vachellia cornigera is a pioneer species. Juvenile individuals that are found in shaded forest habitat tend not to grow large enough to reach the canopy. Bullhorn acacias that are 5-100 cm tall may be found growing in heavy shade but are expected to be unoccupied by ants because of their lack of healthy swollen thorns and food resources (Coelho et al., 2009). Acacias that are 100-250 cm tall can be found in canopy gaps and may be able to attract *P. ferrugineus* with a greater abundance of swollen thorns and a moderate supply of both nectar and Beltian bodies, although, these food sources are of a lower quality on trees of this size class. If younger acacias do not reach the forest canopy they disappear within three years. In a growing population of *V.*

cornigera, individuals that are the tallest or help to form the forest canopy will be occupied by colonies of *P. ferrugineus* (Coelho et al., 2009, Janzen 1967b). Canopy members of *V. cornigera* that are occupied by *P. ferrugineus* benefit most from the ant colony's protection of growing shoot tips (Janzen 1967b). The ant community occupying a population of *V. cornigera* can be used as a bioindicator of the conditions of the acacias and the habitat in general as ants are sensitive to environmental change (Majer, 1983; 1986).

It is common in disturbed habitat to find bullhorn acacia populations comprising all life stages, with the most abundant size class being the smallest seedlings that are found near reproductive adults (Janzen 1967b). In habitat that lacks heavy cattle grazing, it is also common to find all occupied individuals belonging to the largest size class. Increased grazing pressure would open up the forest habitat, exposing the smallest acacia individuals to sunlight which allows for growth to maturity. Seedlings (10-50 cm) of reproductive acacias can be scattered throughout the habitat occupied by their parents but die after 1-2 years if they are not colonized by obligate acacia-ants (Janzen, 1974). Once colonized by a founding queen ant, it takes 9 months or more for a colony to grow large enough to protect their host acacia.

Gove et al. (2005) conducted a study on ant community structure in secondary regrowth and isolated pasture trees and found that estimated species richness of arboreal ants was low on isolated trees, and that neither secondary regrowth nor isolated trees supported as many ant species as small forest fragments. Isolated trees in pasture do, however, support species of *Pseudomyrmex*. *Vachellia cornigera* thrives in disturbed habitat like pasture and secondary

forest, and the lack of species richness in the ant communities of these habitats could lead to the dominance of one or a few species.

When multiple acacia-occupying species within *Pseudomyrmex* are present in the same habitat, competition for acacia hosts will occur. This is because nest sites are a limiting resource and each acacia can typically only host one species at a time (Campbell et al., 2015). However, competition may occur on one acacia individual, where multiple species will occupy different branches of the acacia. This was observed by Clement et al. (2008) on acacias where larger colonies of *P. ferrugineus* dominated the upper branches of larger trees while smaller colonies of *P. gracilis* and other species occupied the lower branches and the lower trunk. Sanchez & Rico-Gray (2011) have also found *P. ferrugineus* and *P. gracilis* co-occupying *V. cornigera* in the tropical deciduous forest of Mexico, where *P. ferrugineus* colonized larger acacias than *P. gracilis*. Coelho et al. (2009) found that *P. gracilis* only occupied trees in the intermediate life stage in a habitat dominated by *Myracrodruon urundeuva*.

The species of ant that does end up occupying an acacia individual will influence the level of herbivore damage that the host acacia experiences. This is observed in the rate of growth between occupied and unoccupied acacias, which indicates the effectiveness of the residing ant colony in protecting their host tree from herbivorous insects (Janzen, 1967b). Agrawal & Rutter (1998) created a list of cues that may potentially influence an ant colony's anti-herbivory defense, including colony size, density of conspecific worker ants, disturbance, plant tissue damage, food body production, nectar production, presence of herbivores and even mammalian breath. Davidson & McKey (1993) suggest that rate of colony development, colony size and

worker activity level are all factors that affect the protection provided by an ant colony. However, herbivory defense seems to level off when an ant colony has greater than 300 individuals (Rocha and Bergallo, 1992). Colonies of *P. ferrugineus* grow to be much larger than those of *P. gracilis*, and since *P. ferrugineus* is an obligate acacia-ant species its colonies aggressively defend their host acacias, while colonies of *P. gracilis* do not typically exhibit aggressive behavior while occupying acacias.

A mutualistic relationship can only be maintained if the benefits of the mutualism, such as the identity of the partner species and need for a mutualist, outweigh the costs, such as production of food resources for colonizing ants (Bronstein, 1998). In the case of the mutualism between *V. cornigera* and *P. ferrugineus*, other conditions of the habitat, including nutrient and light availability and herbivore abundance, can influence the need to maintain the mutualism. Pringle et al. (2012) found that larger individuals of *Cordia alliodora* experience higher herbivore pressure per leaf area than smaller individuals, and that herbivory per unit leaf area increased exponentially with tree size when its mutualistic *Azteca pittieri* ants were experimentally excluded from leaves. A lack of occupation by a mutualistic ant colony results in a decrease in production of expensive food resources such as food bodies and nectar (Agrawal and Rutter, 1998). The occupancy of bullhorn acacias in a habitat, or lack thereof, may reflect the conditions of the habitat. In restored forest habitat, where conditions may be stressful even for pioneer tree species depending on the land-use history of the habitat, ant occupancy could serve as an indicator of the conditions of the habitat as the bullhorn acacia will invest resources into attracting obligate acacia-ants in response to its conditions.

B. Methods

1. Ant Occupancy Observations

Data was collected May 15-19, 2017. The presence or absence of occupying ants was determined for each recorded acacia individual. For those acacia individuals that had ants present, the species was identified — as either *Pseudomyrmex ferrugineus* (Smith, 1877) or *Pseudomyrmex gracilis* (Fabricius, 1804) — as these were the only two ant species found on the acacias. Ant occupancy is here defined as the residence of ant individuals in a colony, disregarding the size of the colony, in the thorns of an acacia. Evidence of ants taking up residence on an acacia is provided by the presence of entrance holes cut into the acacia's thorns and the movement of ants in and out of such thorns.

Observations of ant occupancy were made between 10-3pm, where the beginning of this time period (10-12pm) is the peak of ant activity when they are expected to be moving on their host acacia to patrol and/or forage (Raine et al., 2002). The presence of ant occupants on an acacia was determined by both the presence of entrance holes and individual ants on the plant at the time of observation. Acacia individuals that did not initially have active ants on the plant surface were agitated to simulate herbivory and stimulate defensive (in the case of *P. ferrugineus*) or evasive (in the case of *P. gracilis*, where they may move off of the acacia entirely) behavior. The appearance of a minimum of two ant individuals was taken to indicate that ants were occupying the acacia. The presence of entrance holes alone does not provide sufficient evidence of current occupancy of an acacia by ants, as the acacia could have been abandoned. Ant occupancy data were collected for 127 of the recorded acacia individuals.

Ant species were identified according to distinctive physical characteristics. *Pseudomyrmex ferrugineus* is an obligate acacia-ant belonging to the *Pseudomyrmex ferrugineus* group, along with nine other species. *Pseudomyrmex ferrugineus* (Figure 9) is smaller (HW 0.92-1.15 for workers) and is a light brown color (Ward, 1993). More specific identifying characteristics for *P. ferrugineus* include: common pilosity on the mesonoma ($MSC > 10$), six teeth on the masticatory margin of the mandible, sparse tibial pilosity ($HTC + MTC < 8$) and short eye size (REL 0.36-0.46). *Pseudomyrmex gracilis* is one of six generalist *Pseudomyrmex* species that occupy swollen-thorn acacias opportunistically. *Pseudomyrmex gracilis* (Figure 10) is larger (HW 1.39-2.07 for workers) and has a distinctive color pattern on its body where its head and abdomen are black and its thorax is orange and black (Ward, 1993). Its identifying characteristics include: 7-10 teeth on the masticatory margin of the mandible, a rounded median clypeal lobe, common mesonoma and tibial pilosity ($HTC + MTC > 10$), elongate eyes (REL 0.48-0.68) and a laterally margined pronotum. While ant species were easily identifiable in the field, voucher specimens were collected (two for *P. gracilis* and one for *P. ferrugineus*) to confirm identification.



Figure 9. Photos of *P. ferrugineus* (front, lateral and dorsal views; provided by antweb.org with permission from P.S. Ward)



Figure 10. Photos of *P. gracilis* (front, lateral and dorsal views; provided by antweb.org with permission from P. S. Ward)

2. Herbivory Estimation

Photos were taken of 117 of the recorded acacia individuals to estimate percent herbivory damage. Percent herbivory damage was estimated from three bipinnate leaves for each acacia individual, whether from three different photos or from multiple leaves in one photo. Nine acacias only had photos of two leaves from which herbivory could be estimated and three acacias only had one leaf that could be used in percent herbivory estimation. Leaves on which the flat surface of each pinnule was clearly visible were used.

Percent herbivory was divided into five categories: 0 = 0% herbivory damage, 1 = 1-25% herbivory damage, 2 = 26-50% herbivory damage, 3 = 51-75% herbivory damage and 4 = 76-100% herbivory damage. Herbivory rankings were determined for each primary leaflet and then averaged for the entire bipinnate leaf (Figure 12). Damage was considered to be the proportion of pinnules, or secondary leaflets, with partial or complete removal. Missing primary leaflets, where the stem as well as any pinnules were missing, were not considered in the herbivory estimate, as the removal of entire primary leaflets may have been caused by disturbances such as human or

cattle movement and the number of missing pinnules could not be estimated with confidence.

The rankings of three bipinnate leaves were averaged to get an overall herbivory ranking for each acacia. New growth, which has a distinct bright green color, was not considered.



Figure 11. Labelled photo of one bipinnate leaf with moderate herbivory damage belonging to *V. cornigera*

3. **Statistical Analysis**

A generalized linear model was used to determine the influence of restoration treatment on acacia occupancy status (no ants, occupied by *P. gracilis* or occupied by *P. ferrugineus*). A Fisher's exact test for count data was used to determine the effect of acacia location (interior, edge, outside edge) on occupancy status. A chi-square test was used to determine the effect of acacia height class, where eight height classes were used, on occupancy status. A value of 1 was added to each data point for herbivory ranking to eliminate values of 0, then herbivory data was square root transformed and analyzed with a one-way ANOVA test.

C. **Results**

A total of 86 *V. cornigera* individuals were occupied by ants. *Pseudomyrmex ferrugineus* occupied 27 individuals and *P. gracilis* occupied 59 individuals. Forty-one acacia individuals were not occupied, and the occupancy status could not be confirmed for four individuals.

The effect of restoration treatment on the occupancy status of acacias was not significant, although it appears that a smaller proportion of the acacias growing in the control subplots were unoccupied (21% compared to 31% in animal subplots and 41% in wind subplots) and a slightly larger proportion of the acacias in the animal subplots were occupied by *P. ferrugineus* (29% compared to 21% in control subplots and 13% in wind subplots) (Figure 12).

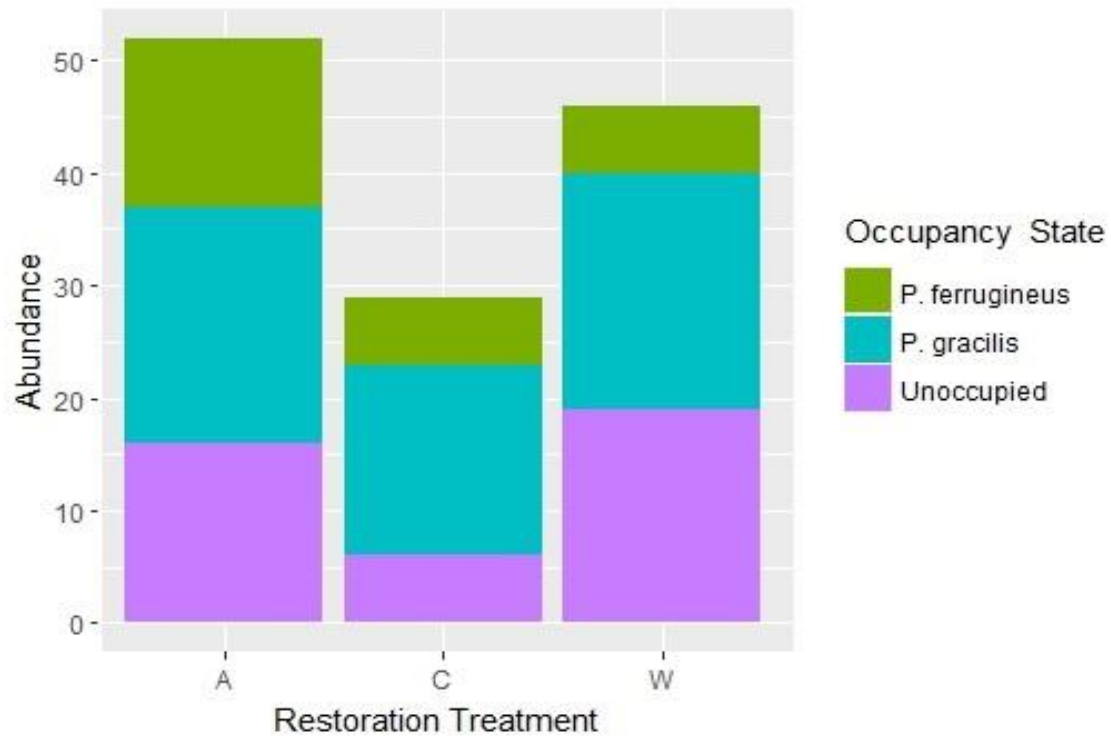


Figure 12. Stacked barplot of total abundances of *V. cornigera* individuals in each occupancy state within each restoration treatment (A = Animal, C = Control, W = Wind)

The results of a Fisher's exact test for count data demonstrate that the location of acacias within plots had a significant effect on their ant occupancy ($p=0.03505$) (Figure 13). The total number of occupied acacias was much higher (58 individuals) at the edge of the subplots than in the interior (8 individuals) or outside the edge of the subplots (20 individuals). Acacias at the edge of subplots hosted the greatest proportion of *P. gracilis* (69%) and *P. ferrugineus* (63%), although 37% of all acacias found in the interior of subplots were occupied by *P. ferrugineus*, compared to only 21% of acacias at the edge and 14% outside the edge.

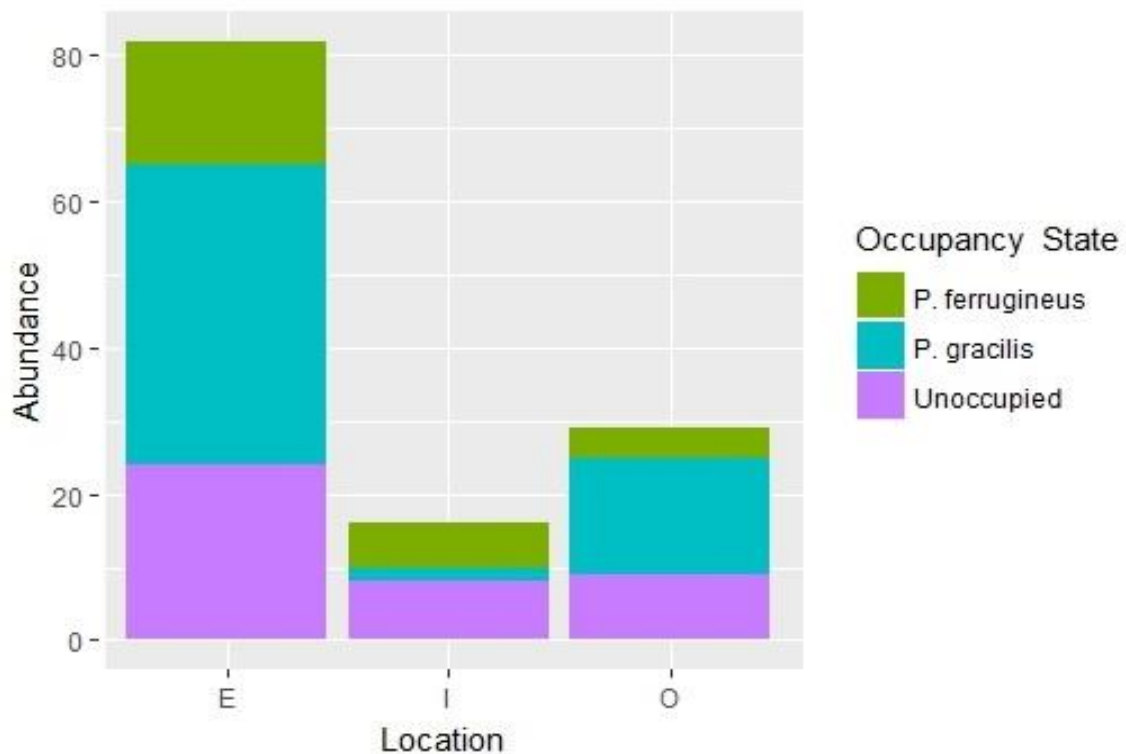


Figure 13. Stacked barplot of total abundances of *V. cornigera* individuals in each occupancy state within each location (E = Edge, I = Interior, O = Outside Edge)

There was a significant effect of *V. cornigera* height on occupancy state ($X^2=80.32$, $df=14$, $p<0.01$), where acacias that were not occupied by ants ranged between 23-107 cm, acacias occupied by *P. gracilis* ranged between 18-500+ cm (where the greatest proportion of these acacias were 30-60 cm) and acacias occupied by *P. ferrugineus* ranged between 64-500+ cm (where the greatest proportion of these were 170-500+ cm) (Figure 14).

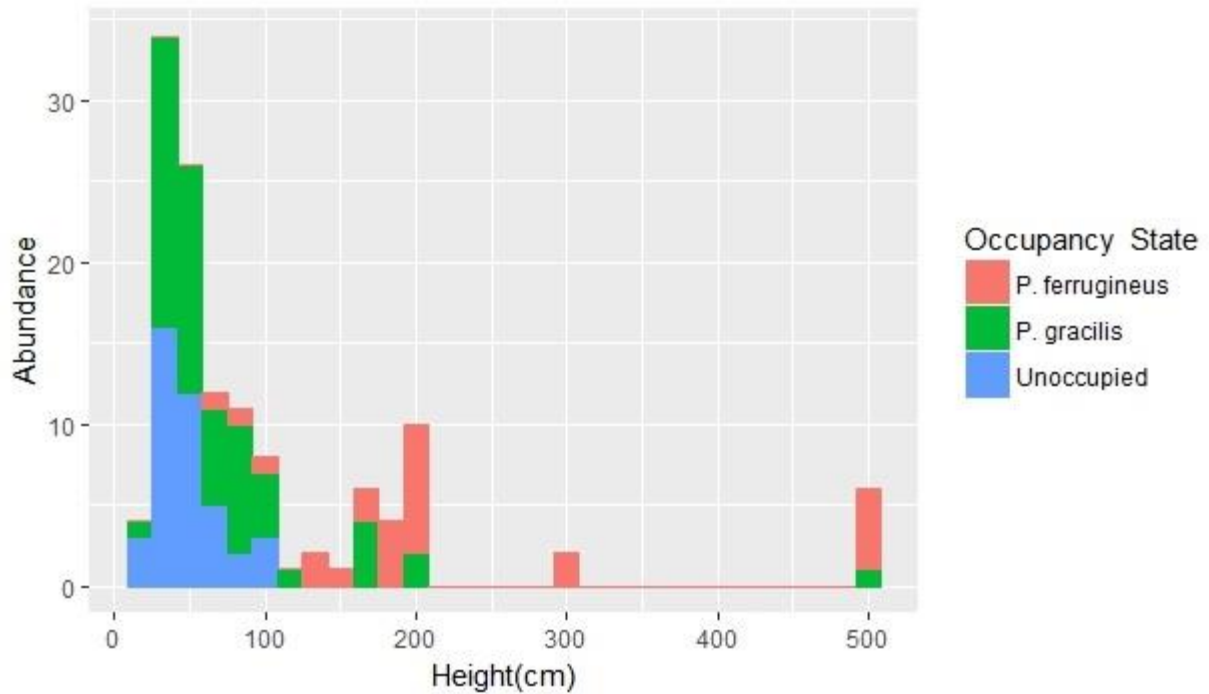


Figure 14. Histogram of *V. cornigera* by height (cm) color-coded according to the occupancy state of each *V. cornigera* individual

The effect of ant occupancy type on the level of herbivory damage incurred on the acacias was statistically significant ($F_{2,110}=25.19$, $p<0.001$). The average herbivory damage ranking was greater than rank 2 (26-50% damage) for both unoccupied acacias (2.42 ± 0.21) and acacias occupied by *P. gracilis* (2.27 ± 0.15), whereas it was less than rank 1 (1-25% damage) for acacias occupied by *P. ferrugineus* (0.67 ± 0.19) (Figure 15). It appears that acacias occupied by *P. gracilis* experience slightly less severe herbivory damage than those that are unoccupied.

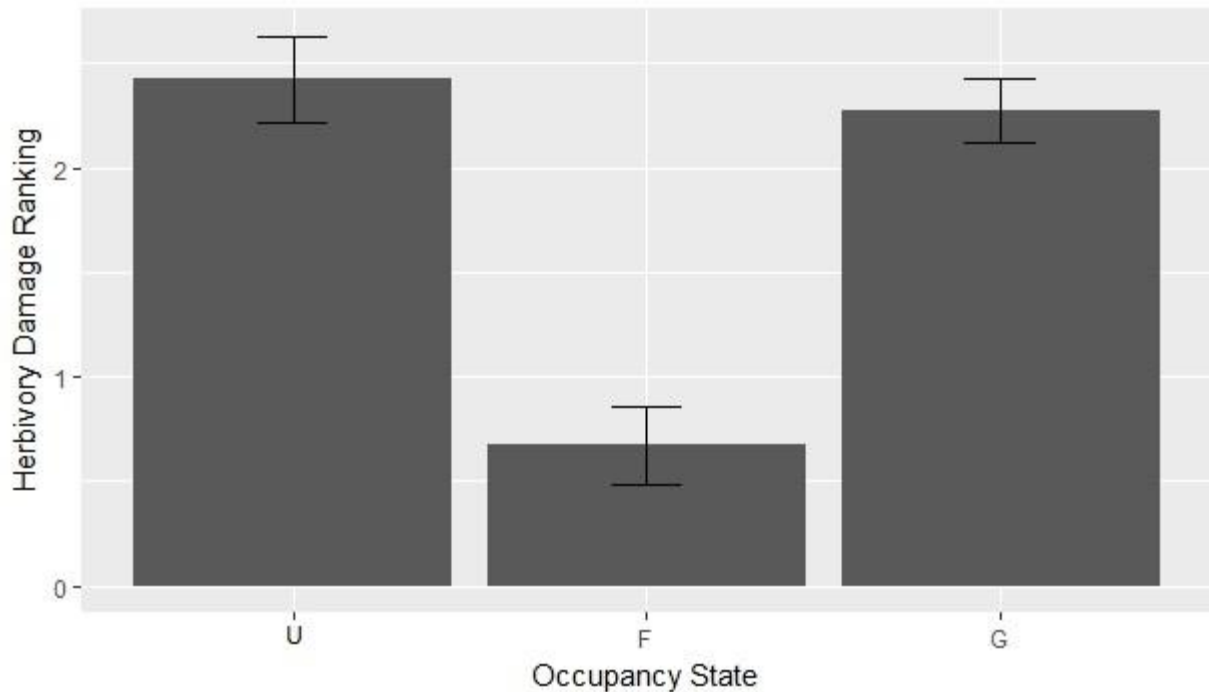


Figure 15. Barplot of mean herbivory damage ranking (0-4) for *V. cornigera* individuals by their occupancy state (U = Unoccupied, F = *P. ferrugineus*, G = *P. gracilis*) with standard error bars

D. Discussion

It appears that an edge effect occurs within each of the forest plots at the restoration site. The edge of the plots is functioning as a transition zone that hosts the greatest abundance and size-class range of *V. cornigera* individuals. The edge habitat also appears to be favorable for both ant species occupying *V. cornigera*, although most of the acacias growing at the edge of the subplots were occupied by *P. ferrugineus* while the smaller acacias growing just outside the edge were more likely to be occupied by *P. gracilis*. Gove et al. (2005) also found that isolated trees occurring in the transition habitat between pasture and forest hosted a high density of ant species, as species typically found in either habitat type can also occur in the transition zone. It is

expected that the species richness of arboreal ants decreases within the interior of forests because the canopy cover decreases the amount of sunlight reaching the ants, and species that are found in open habitat do not do well in shaded, cooler habitat (Queiroz and Ribas, 2016). However, an increase in tree density within the interior of forests provides more habitat and food resources for an ant community, allowing for the co-existence of more species (Ribas et al., 2003).

Pseudomyrmex gracilis is a generalist species that can occupy *V. cornigera* opportunistically, but is able to nest elsewhere, such as inside twigs, if individuals of *V. cornigera* are not available for colonization. The smallest proportion of acacias occupied by *P. gracilis* occurred in the interior of the subplots, where shaded conditions may not be ideal for this disturbance-tolerant species. *Pseudomyrmex ferrugineus* appears to be more capable of occupying acacias growing in the interior of subplots. Both ant species flourished at the edge of subplots, where the highest proportion of *P. ferrugineus*-occupied acacias occurred, while *P. gracilis* dominated the acacias growing outside the edge of the plots. These occupancy patterns may demonstrate the tolerances of each ant species to different habitat conditions, but the edge seems to be ideal for both.

Wherever *P. ferrugineus* and *P. gracilis* are found to co-exist in the deciduous tropical forest of Mexico, *P. ferrugineus* occupies larger individuals of *V. cornigera* than *P. gracilis* (Sanchez & Rico-Gray 2011). This could be a form of niche partitioning between the two species, or just competitive exclusion by *P. ferrugineus*. Philpott (2010) found that the presence of *Azteca instabilis*, a dominant ant species inhabiting the forest canopy of a coffee agroecosystem, decreased the rate of colonization by a generalist twig-nesting ant species but

that an increase in tree density counteracts this competitive exclusion. Ant species may form a distribution of territories within habitat that consists of long-lived trees and shrubs (Jackson, 1984; Adams, 1994). Although *V. cornigera* is a pioneer species that is relatively short-lived, it can dominate secondary succession such as our restored forest plots in the early years of succession. Jiminez-Soto & Philpott (2015) have found that *P. gracilis* more often occupies coffee shrubs than canopy shade trees within forest, which could indicate their preference for more open habitat.

There is a distinct relationship between *V. cornigera* height and ant occupancy status at the study site, where the smallest acacias do not host any ants, the intermediate size classes are occupied by *P. gracilis* and the largest size classes are occupied by *P. ferrugineus*. While both species were never found to occupy the same acacia as was observed by Clement et al. (2008), competition between the ant species may be occurring within the population of acacias at the study site. *Pseudomyrmex ferrugineus* outcompetes *P. gracilis* for the largest acacias in the forest subplots, so *P. gracilis* occupies smaller acacias at the edge of the subplots and the smallest acacias growing just outside the plots. Both location and acacia height are influencing the distribution of the ants, where *P. ferrugineus* is colonizing larger acacias mostly at the edge and also in the interior and *P. gracilis* is occupying smaller acacias at the edge and outside the plots where more sunlight is available to them.

Occupancy by *P. ferrugineus* reduced the level of herbivory damage incurred by *V. cornigera* individuals. Fonseca (1994) observed that another myrmecophytic tree species, *Tachigali myrmecophila*, attracted greater than 4 times more herbivorous insects and experienced

a rate of herbivory ten times higher when not occupied by *Pseudomyrmex concolor*. Plants were especially vulnerable to herbivory in the wet season and if they had immature leaves.

An interesting result from this study is that *V. cornigera* individuals that were occupied by *P. gracilis* experienced less herbivory damage overall than plants that were not occupied at all. The population of *P. gracilis* ants in the restoration site did not demonstrate aggressive behavior at all, but Agrawal and Rutter (1998) point out that observations of passive behavior alone are not indicative of how these ants may be defending their host plants. Obligate acacia-ants occupying plants of the *Vachellia* genus evolved from “timid” ancestors that inhabit dead twigs, much like *P. gracilis* (Ward and Branstetter, 2017). Since *P. gracilis* was mostly occupying *V. cornigera* individuals at the edge and outside the edge of the subplots where there is greater herbivory pressure, they may facultatively demonstrate aggression towards herbivorous insects to protect those acacias within their competitive range.

The restoration site provides an opportunity to study the interactions between *V. cornigera* and the ant species that occupy it in a unique environment. The restored-forest plots are isolated from larger fragments of primary and secondary forest by being located within an active cattle pasture, and each plot is fenced to protect it from cattle grazing. Acacias within the interior and, to a lesser degree, at the edge of the plots may experience reduced herbivore pressure. If this is the case, the costs of maintaining the resources required to attract mutualistic ant colonies may outweigh the need for their protective services. Evidence for a lack of investment in attracting ant colonies by *V. cornigera* was observed at the study site in the absence of Beltian bodies on all but a couple of the largest acacias. This may indicate that the

acacia population is either experiencing stressful habitat conditions and cannot invest resources to produce Beltian bodies, or do not need to produce Beltian bodies because herbivory pressure is not great enough to warrant it. Overall herbivory damage of observed acacias was rather low, with an average herbivory ranking of less than 3 (51-75% damage) on a scale of 0-4 for all three occupancy states. Janzen (1974) came across an island population of *V. cornigera* that was released from pressure by herbivorous insects and therefore did not require occupation by obligate acacia-ants for survival. A lack of herbivorous insects and competition by other early-successional plant species in disturbed habitat like cattle pasture may allow small unoccupied acacias to persist for many months (Janzen, 1975). These smaller acacias are prime habitat for exploitative ant species like *P. gracilis*. The nectar-secretion rates of acacias was not measured, but this could be another indicator of their response to herbivory levels or habitat conditions.

It appears that the edge of the restored forest subplots is ideal for both the recruitment of a greater abundance and size-class range of *V. cornigera*, and in effect has also become ideal habitat for both ant species occupying *V. cornigera* at the study site. The greater abundance of acacias at the edge of subplots allows for *P. ferrugineus* and *P. gracilis* to co-exist on the same population of acacias, and the range of *V. cornigera* size classes occurring at the edge habitat has led to a form of niche partitioning between the two ant species, where *P. ferrugineus* occupies the larger acacias and *P. gracilis* occupies the smaller acacias. The dominant-subordinate relationship between these two species has been documented for individual acacias as well as populations of acacias of varying sizes, but this study has demonstrated that habitat conditions may also influence the dynamic between the two species. *Pseudomyrmex gracilis* is a disturbance-tolerant generalist that is able to occupy acacias growing at the edge and outside the

edge of the forest subplots, while *P. ferrugineus* dominates larger acacias that are found at the edge and in the interior of the subplots. While the interior is more shaded, a larger size class of acacias is growing there. The smallest acacias are growing outside the edge of the subplots. *Pseudomyrmex ferrugineus* can occupy acacias that are canopy members of forest or smaller acacias growing in canopy gaps. Overall herbivory stress at the study site is on the low side, which could be the reason why such a large proportion of the acacias were occupied by *P. gracilis*, although these acacias suffered greater herbivory damage than those occupied by *P. ferrugineus*. Unoccupied acacias fell within the smallest size classes and were mostly growing outside the subplots. Their lack of occupancy could either reflect a lack of herbivory pressure or the preference and opportunity for *P. gracilis* and *P. ferrugineus* to occupy larger acacias with more and better food rewards. In the context of restored forest habitat, the interactions between *V. cornigera* and its occupying ants can indicate the conditions of the habitat in terms of herbivory pressure, edge effects and acacia recruitment history.

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APPENDIX

TABLE I. GENERALIZED LINEAR MODEL RESULTS FOR EFFECT OF RESTORATION TREATMENT ON *V. CORNIGERA* ABUNDANCE

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.1468	-0.8227	-0.4385	0.2623	1.9889

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.9459	0.2843	6.845	9.11e-07 ***
data\$Plot_TreatmentC	-0.6581	0.4274	-1.540	0.139
data\$Plot_TreatmentW	-0.1967	0.4081	-0.482	0.635

(Dispersion parameter for Negative Binomial(2.7214) family taken to be 1.266752)

Null deviance: 28.662 on 23 degrees of freedom
Residual deviance: 25.588 on 21 degrees of freedom

AIC: 134.53

TABLE II. GENERALIZED LINEAR MODEL RESULTS FOR EFFECT OF PLOT LOCATION ON *V. CORNIGERA* ABUNDANCE

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.7639	-1.0907	-0.4453	0.2166	3.1389

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.2408	0.2702	4.592	1.92e-05 ***
data\$Location_by_PlotI	-1.4744	0.4526	-3.257	0.00175 **
data\$Location_by_PlotO	-1.0515	0.4226	-2.488	0.01525 *

(Dispersion parameter for Negative Binomial(1.0877) family taken to be 1.450115)

Null deviance: 95.139 on 71 degrees of freedom
Residual deviance: 76.082 on 69 degrees of freedom

AIC: 253.88

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